Methods for Assessing the Effects of Chemicals on Reproductive Functions
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1 Introduction, General Conclusions and Recommendations*

1.1 INTRODUCTION

The interest in and importance of both human and non-human reproduction are obvious. In both of these subjects there are complexities and conflicting elements.

The reproduction of many non-human species is of economic importance to human beings and necessary for their nutrition. Obvious examples are domestic animals, fish and cereals. In some cases the food is actually a part of the reproductive process, e.g. eggs and seeds.

Other examples of human dependence on non-human populations are intestinal bacteria and pollinating insects of which it is important to have an appropriate kind and quantity.

On the other hand, human beings may wish to reduce the reproduction of populations that interfere with their health or well-being, for example: vermin that eat human food or carry disease; biting insects; insects that carry disease; plants that overgrow waters or soil; and pathogenic bacteria.

In addition to these homocentric considerations there are important ecological implications of non-human reproduction. The condition of an ecosystem depends on the rate of reproduction of a multitude of species in relation to the death rate of each species and to the interactions between species. The relations between species and ecosystems are complex and largely unknown. The extermination of a species represents an irretrievable loss and, since its long-term consequences cannot be predicted, man's activities should not be permitted to have this result.

In the present report the discussions of methods for evaluating the effects of chemicals on reproductive functions are divided into two parts, one dealing with mammalian, the other with non-mammalian targets. This seemed justified because of the appreciable differences in basic concepts and experimental approaches. Although there was some criticism of the approximately equal space and attention being devoted to the two groups of biota, considering the enormous variety and number of phyla (divisions), classes, families, genera and

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species, and the innumerable individuals in the non-mammalian group of animals, plants and microorganisms in comparison with one class of mammals, it was manifestly impossible to allocate attention on this basis.

Another justification for the choice of the subject for this SGOMSEC study arises from the notable lack of methods for assessing the effects of chemicals on reproductive function; additionally, such methods are rarely included in a standard battery of toxicological tests, and if so, only to a limited extent. It appeared during the discussions in this Workshop that for many reproductive functions, especially human, the tests consisted of qualitative assessment of morphology rather than of quantitative methods for measuring specific biological functions.

The Workshop concentrated on test procedures that would assist scientists in giving advice to governmental control and regulatory agencies, industries and research-supporting organizations. It was assumed that these scientists know most of the science basic to the methodology. For this reason the reader will find only a minimum coverage of this material in the Joint Report which concentrates as far as possible on the tests of the effects of chemicals on reproduction where they exist, their suitability, their shortcomings and, possibly, their lacks. In the assessment of the known tests, a number of attributes were considered including the following.

Relevance to practical problems As far as possible methods should be suitable for assessing and illuminating problems involving chemicals, species and effects that have created difficulties and may do so again. An example that comes to mind is the effect of accumulated chlorinated hydrocarbons on the reproduction of birds of prey.

Economy Wherever possible the procedures should not require too much expenditure of effort and money, especially if they are to be routinely and widely used. Frequently, some compromise with this principle will be required. For example, *in vivo* laboratory tests may be the cheapest, but expensive population investigations under field conditions may be considered the most relevant. A serious limitation in studies of large mammals is that the number of individuals studied is frequently limited to samples too small to give statistical confidence.

Predictive ability It is difficult to decide *a priori* which tests may lead to confident predictions. However, a few generalizations are possible. As mentioned above, population studies of effects under field conditions are often the most relevant. If tests can be found that are valid for a whole class of chemicals, they are likely to be more efficient and economical. To assist prediction, tests should permit quantitative assessment of the response to graded dosing. There are many difficulties in these quantifications. In epidemiological studies the doses are rarely if ever known, even approximately, and, as mentioned above, many histological effects are described without quantification.

Another problem of quantitative testing is the difficulty of knowing how long

to continue the exposure, how to express it (concentration × time, concentration in target tissue, amount absorbed systemically, etc.) and how long to continue observing the response (hours, days, a lifetime, more than one generation). Nevertheless it is the dose and the relative sensitivity of the receptor to a chemical that determine the relative hazard.

Acceptability This concept applies to a method that may find general acceptance because it is relevant to practical problems, is economical, has predictive ability, is easy to perform, and gives comparable results. The advantage of widely accepted methods performed uniformly and with widely accepted results is obvious. Many such tests exist already, with or without validation. Where possible these are listed, described and assessed.

Reservations were generally expressed about the applicability of tests on a single part of the reproductive process, performed on a single species with a single pure chemical. In real life several toxins or stresses may be operating simultaneously and there may be considerable interspecies reactions such as competition and predation.

Methods for testing the reproductive effects on mammals are discussed in two sections of the Joint Report, one dealing with females, the other with males. The section on females includes integrated reproduction studies which reflect the overall reproductive capability of the mammalian species, and a discussion of epidemiological investigations applicable, in principle, to both the female and male function. This is followed by a section on methods for testing the effects of chemicals on vertebrates other than mammals, separate subsections being devoted to birds, fishes, reptiles, and amphibians. The report on invertebrates is divided differently: one subsection is devoted to field studies and analyses of field-collected material, the other to laboratory studies covering Platyhelminthes, Cnidaria, Aschelminthes, Annelida, Arthropoda, Mollusca, and Echinodermata. In the last section, methods applicable to higher plants, algae and microorganisms are reviewed. Lists of references cited in the Joint Report are attached to each section and so are the specific conclusions and recommendations for further development of methods and for the methodological research required.

1.2 GENERAL CONCLUSIONS AND RECOMMENDATIONS

(1) Toxicology of reproductive function is a rapidly developing field and requires continuous updating. Methods for laboratory testing and field studies discussed in this report need to be reviewed and, if necessary, revised within the next 3–5 years.

(2) National and international scientific organizations should be informed of the conclusions and recommendations of this meeting so that the whole scientific community becomes aware of the areas of greatest priority for research.

(3) Working groups concerned with effects of chemicals on reproductive function of microorganisms, plants and animals other than mammals require an extensive specialized expertise to be able to consider adequately the many species, genera, families, orders, classes and phyla involved. This should be taken into account when convening future workshops.

(4) Within the International Programme on Chemical Safety a system should be developed for international cooperation in the field of chemical effects on reproduction in non-human targets, including domestic and agricultural animals and plants.

(5) An international organized interlaboratory comparison programme should be established to validate the available tests under a variety of conditions. For many species, generally used sets of methods and procedures are available; however, their applicability under different conditions should be verified.

(6) Interlaboratory comparison and validation projects require reference chemicals known to affect reproductive function in different species. An international bank of such chemicals should be established so that samples become readily available, on request, to research laboratories and field stations in every part of the world.

(7) Field studies are necessary for most species of plants and animals in order to ascertain the relationship between effects observed in the laboratory or in individual case studies and effects that arise under real field conditions. They are also required to identify the most sensitive indicator species and species suitable for laboratory studies (test species).

(8) The main problem in field studies, including epidemiological investigations, is the proper assessment of exposure. Without effective methods for estimating exposure such studies are difficult to interpret. This is a problem common to all toxicological field studies.

(9) More effort must be directed towards developing test procedures for assessing reliably, quantitatively, and with precision the fate and effects of chemicals within defined ecosystems.

(10) When developing tests for effects on reproductive function one should not overlook the possibility of biological conversion of chemicals into more toxic forms.